New strains of bean common mosaic virus (BCMV)

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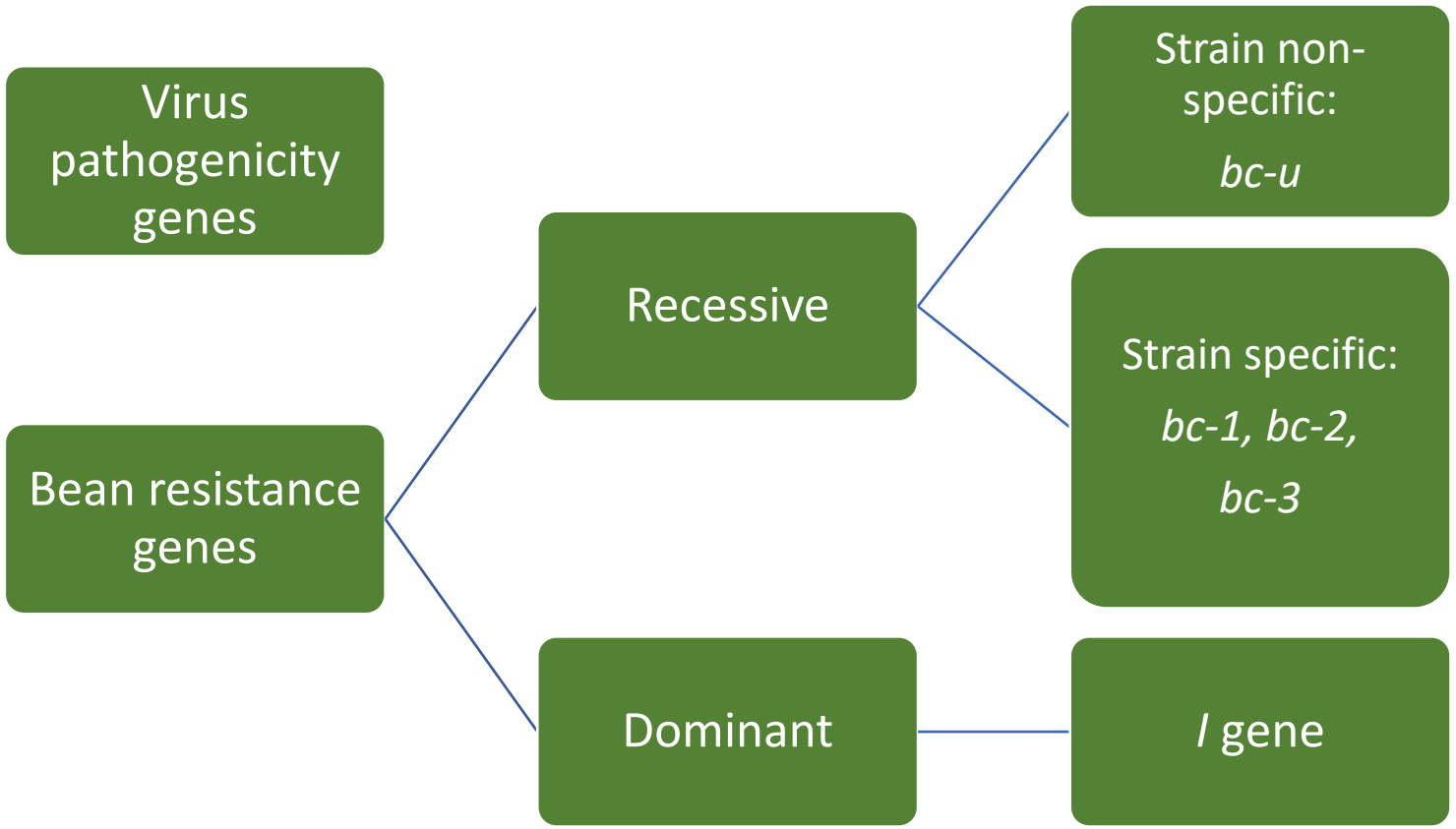
Bean common mosaic virus (BCMV)

- BCMV is a potyvirus, transmitted by aphids in a non-persistent manner
- BCMV represents a world-wide problem in beans
- BCMV is also seed-transmitted in all classes of common beans, with up to 80% efficiency
- Control of BCMV includes seed certification and breeding for resistance

Genetics of BCMV interactions with common beans



Virus functions affected

bc-u: unknown function

bc-1, *bc-2*: long-distance movement

bc-3: replication

I: HR induction

BCMV pathogroups in *Phaseolus vulgaris*

Pathogroup

Differential cultivars	0	I	II	III	IV	V	VI	VII	VIII
DW (*i*)									
SGR (*i*,*bc-1*)									
RGLC (*i*,*bc-u*,*bc-1*)									
RGLB (*i*,*bc-u*,*bc-1²*)									
Sanilac (*i*,*bc-u*,*bc-2*)									
Othello (*i*,*bc-u*,*bc-1²*,*bc-2²*)									
UI35 (*i*,*bc-u, bc-1²*,*bc-2²*)									
IVT7214 (*i*,*bc-u*,*bc-2*,*bc-3*)									
Jubila (*I*,*bc-1*)									
Amanda (*I, bc-1²*)									
US1006 (*I*,*bc-2²*)									
IVT7233 (*I, bc-1², bc-2²*)									

Overview of host ranges for BCMV strains

- Main groups of strains:
  - Common bean (BCMV-US1 and BCMV-RU1) / Azuki bean (AzMV)
  - Soybean
  - Peanut (PStV) / Lima bean
  - Blackeye cowpea (BlCMV)
  - Host ranges and reciprocal effects of BCMV strain groups in different legume hosts are poorly known

Crotalaria micans affected with BCMV

Island of Hawaii

- Five samples were collected in Waimea, HI, in August 2018
- Came from a naturalized *Crotalaria micans* plants exhibiting mosaic, yellowing and growth retardation
- Identified as BCMV based on serology
- We established one of the isolates (BCMV-C2) and fully characterized it

Objectives of the study

- Establish this BCMV isolate in the lab and biologically separate it
- To characterize the BCMV isolate molecularly, through whole genome sequencing
- To elucidate possible functions of the *bc-u* gene in common bean

Experimental approach

Crotalaria micans samples, five plants

- Transferred infectious agent(s) to *Nicotiana benthamiana*, mechanically
- Subjected two *C. micans* samples and one NB receptor plants to HTS
- Identified three viruses in total: BCMV, BYMV, and ClYVV
- Separated BCMV-C2 via single-lesion re-inoculation from *Chenopodium quinoa*

Preliminary results after HTS

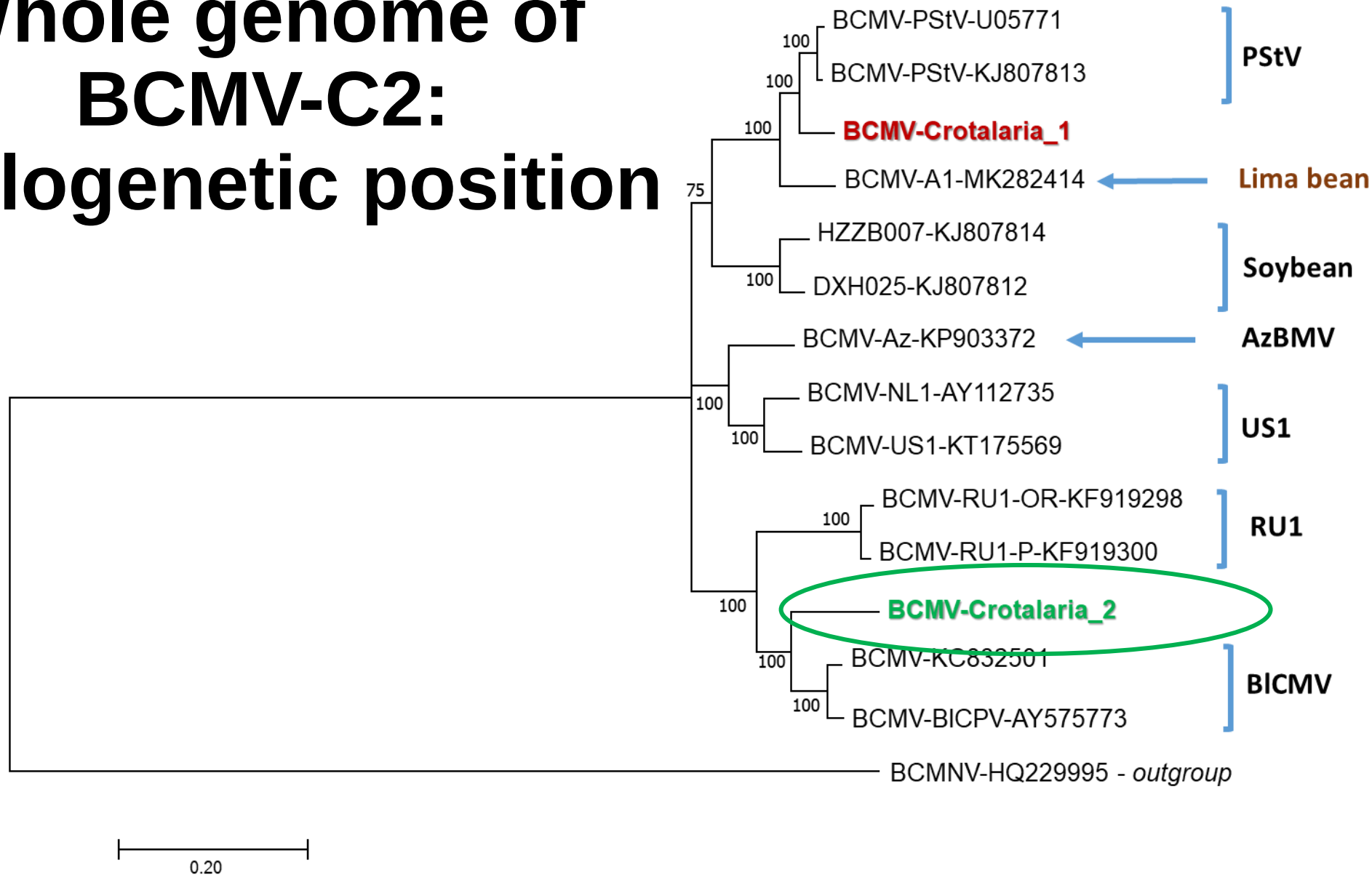
- Two distinct BCMV isolates were found in the original *C. micans* samples :

  BCMV-C1 (close to peanut strain)

  BCMV-C2 (close to blackeye cowpea strain)

- We focused on BCMV-C2, because the blackeye cowpea strain was hypothesized to interact with *bc-u* (J. Myers)

Whole genome of BCMV-C2: phylogenetic position



Rapid decline in common beans without resistance genes, BCMV-C2, 12-dpi

Sutter Pink

Black Turtle II

Dubbele Witte

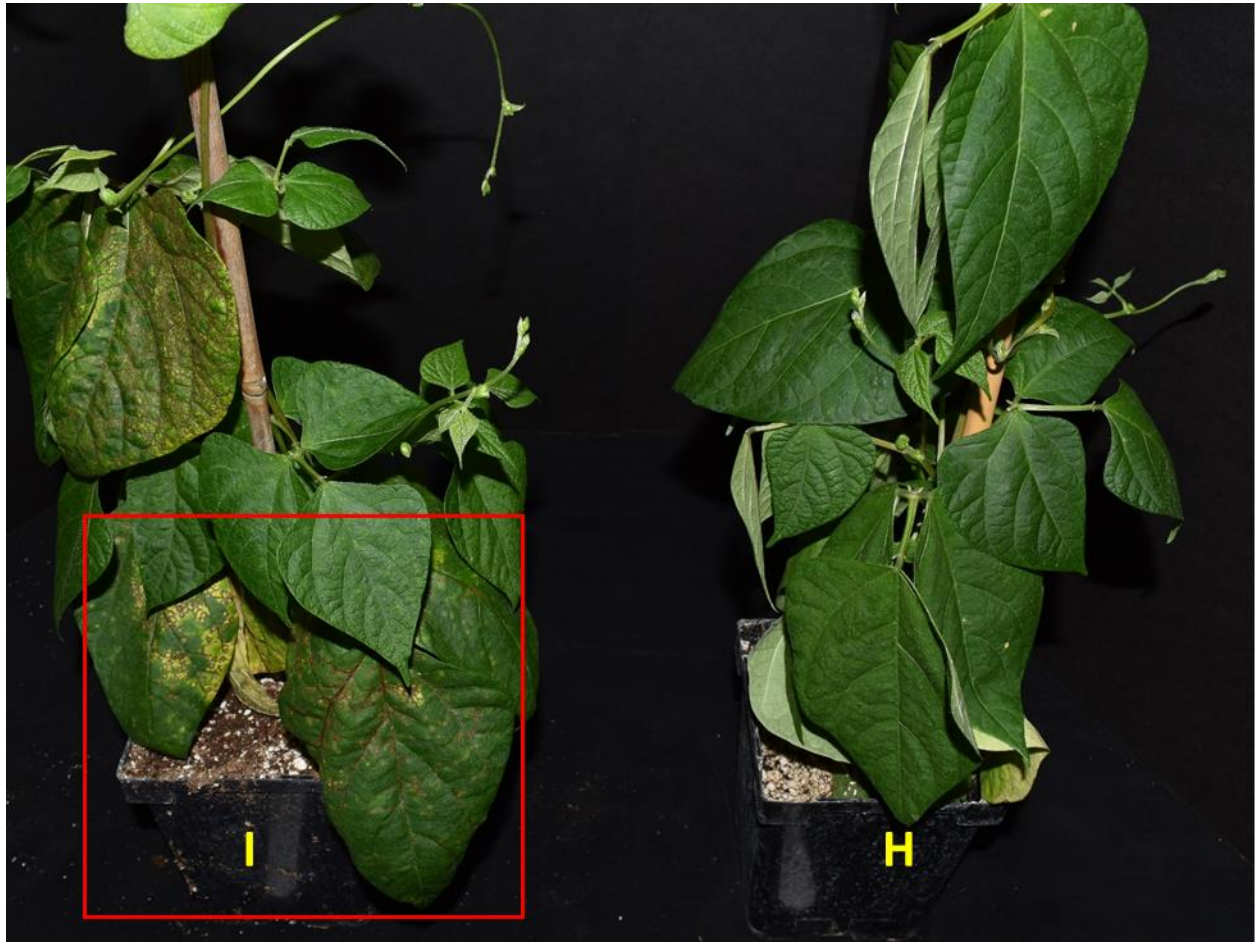
Rapid decline in common beans without resistance genes, BCMV-C2, 18-dpi

Sutter Pink

Black Turtle II

Dubbele Witte

Systemic infection in cv. Bill Z (*bc-u* only), BCMV-C2, 4-wpi



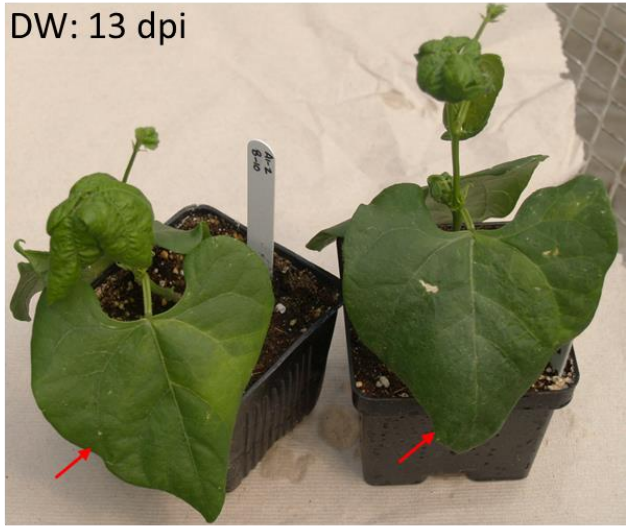
Systemic infection in cv. Poncho (*bc-u* only), BCMV-C2, 4-wpi

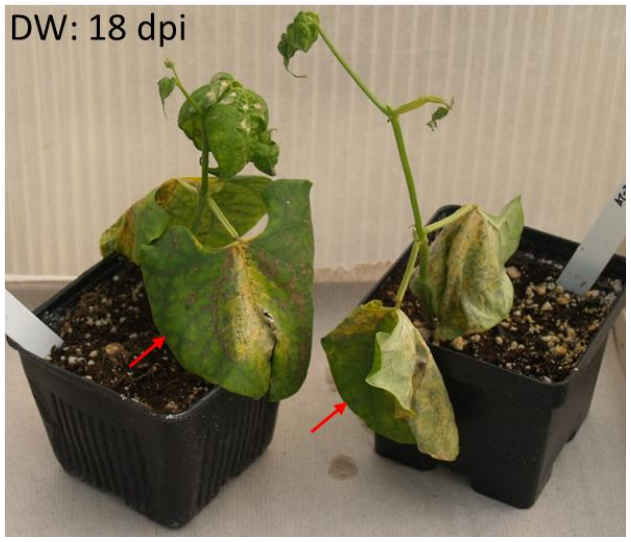
Cultivar/resistance gene(s) [a]	Plants	Inoc. leaves, 12 dpi [b]	Upper, non-inoc. leaves, 8 wpi [b]	Symptoms [c]	
				IL	NIL
DW /none	3	+++	NA	NLL	syst. N, death
Black Turtle II /none	3	+++	NA	NLL	syst. N, death
Pink Sutter /none	3	+++	NA	NLL	syst. N, death
Bill Z /bc-u	3	+++	+++	NLL	M, VN
Buckskin /bc-u	2	+++	+++	NLL	M, VN
Medicine Hat /bc-u	2	+++	+++	NLL	M, VN
Poncho /bc-u	3	+++	+++	NLL	M, VN
UI-228 /bc-u	3	+++	+++	NLL	M, VN
SGR /bc-1	3	+++	-	NLL	NS, NP
RGLC/ bc-1	3	+++	-	NLL	NS
RGLB / bc-1, bc-?	3	+++	-	NLL	NS
Sanilac / bc-2, bc-?	3	+++	-	NLL	NS
UI-35 /bc-u, bc-1, bc-2	3	+++	-	NLL, VN	NS
IVT 7214 / bc-2, bc-3, bc-?	3	-	-	NS	NS
Jubila /I, bc-1	3	-	-	NS	NS
Amanda /I, bc-1	3	-	-	NS	NS
US1006 /I, bc-2	3	-	-	NS	NS
IVT7233 /I, bc-1, bc-2	3	-	-	NS	NS

Summary of BCMV-C2 pathotyping

- BCMV-C2 replicates in inoculated leaves in the absence of *bc-3* and *I* genes
- BCMV-C2 induces local and systemic necrosis (WPN) in the absence of resistance genes
- Presence of *bc-u* prevents WPN but not systemic infection

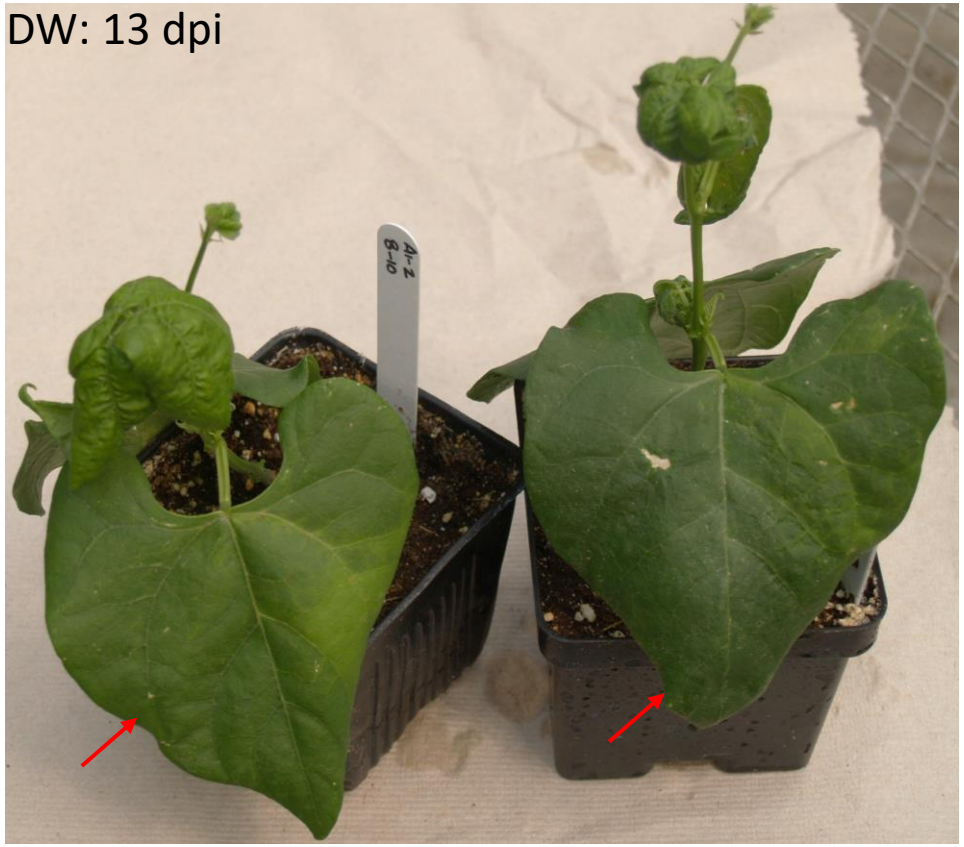
Lima beans affected with BCMV

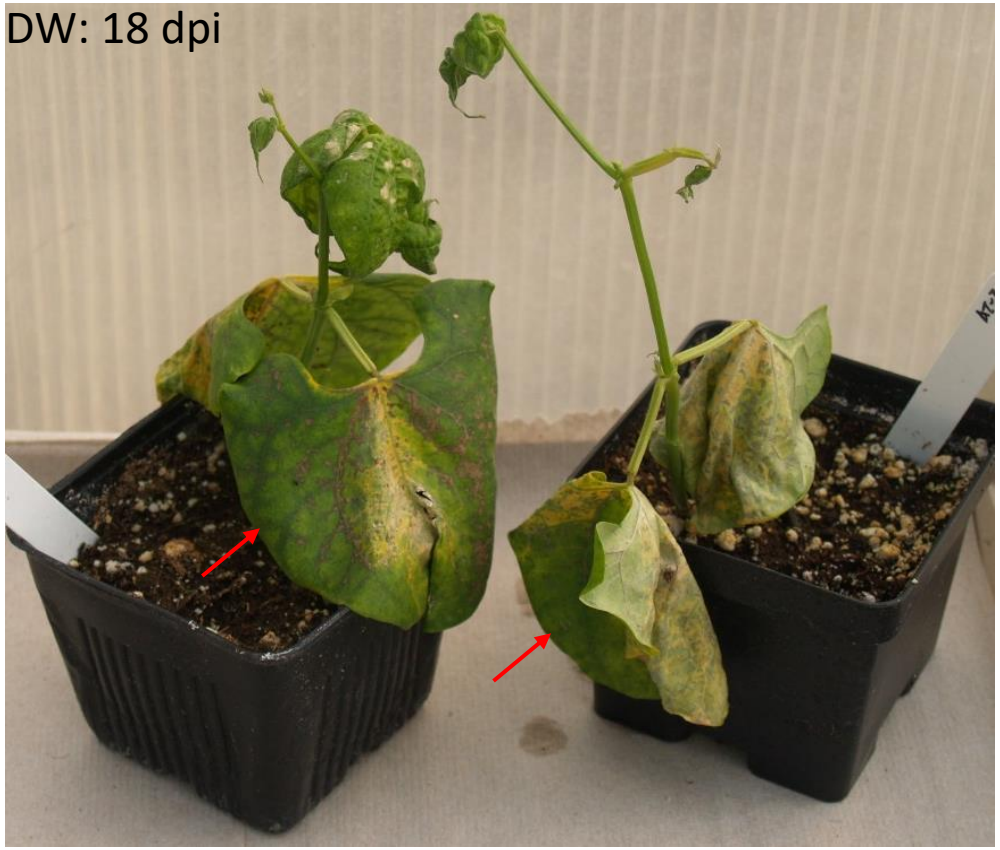




- A sample was collected in Honolulu, HI, in May 2017
- Came from a lima bean (*Phaseolus lunatus*) plant exhibiting mosaic and growth retardation
- Identified as BCMV based on serology
- We established this isolate (BCMV-A1) and fully characterized it

Rapid decline in common bean cv. DW





Summary of biological properties

- BCMV-A1 induced rapid systemic necrosis in ‘Dubbele Witte’ – very unusual
- It induced a less severe systemic necrosis in ‘Stringless Green Refugee’ – very unusual
- It induced mosaic in *Nicotiana benthamiana* – unusual, but convenient
- We used *N. benthamiana* for BCMV-A1 propagation

Summary of the pathotyping on bean differentials

Cultivar/ resistance gene(s)	Plant	Inoculated leaves, 2 wpi		Upper, non-inoculated leaves, 5 wpi		Symptoms	
		Positive/tested	Per cent positive	Positive/tested	Per cent positive	IL	NIL
DW/none	1	2/2	100%	NA	NA	NLL	syst. N
	2	2/2	100%	NA	NA		
	3	2/2	100%	NA	NA		
SGR/*bc-1*	1	2/2	100%	33/34	97%	NLL	MM, VN
	2	2/2	100%	27/27	100%		
	3	2/2	100%	28/28	100%		
RGLC/*bc-u*, *bc-1*	1	2/2	100%	2/24	8%	ClSp	NS
	2	2/2	100%	11/30	37%		
	3	2/2	100%	8/27	30%		
RGLB/*bc-u*, *bc-1²*	1	2/2	100%	18/32	56%	ClSp	NS
	2	2/2	100%	12/24	50%		
	3	2/2	100%	12/25	48%		
Sanilac/*bc-u*, *bc-2*	1	2/2	100%	35/37	95%	ClSp	NS
	2	2/2	100%	30/32	94%		
	3	2/2	100%	6/21	29%		
UI-35/*bc-u*, *bc-1²*, *bc-2²*	1	2/2	100%	0/37	0%	NLL, VN	NS
	2	2/2	100%	0/43	0%		
	3	2/2	100%	0/26	0%		
IVT 7214/*bc-u*, *bc-2*, *bc-3*	1	0/2	0%	0/31	0%	NS	NS
	2	0/2	0%	0/38	0%		
	3	0/2	0%	0/33	0%		

- BCMV-A1 visually has pathotype I
- But based on laboratory tests, it has pathotype VI
- In beans from host groups 2, 3, and 4 systemic infection is asymptomatic
- BCMV-A1 is capable of overcoming two resistance genes, *bc-1* and *bc-2*
- BCMV-A1 represents a new pathotype of BCMV

Justification and objectives

- Why were we interested in this lima bean isolate of BCMV?
- In 2017, Idaho State Department of Agriculture asked us to test common bean samples for the presence of BCMNV
- Samples came from a routine seed certification inspection, from Idaho
- No BCMNV was found – customers were happy
- However, half of the samples were typed as conventional BCMV and about half as a new PStV-like BCMV

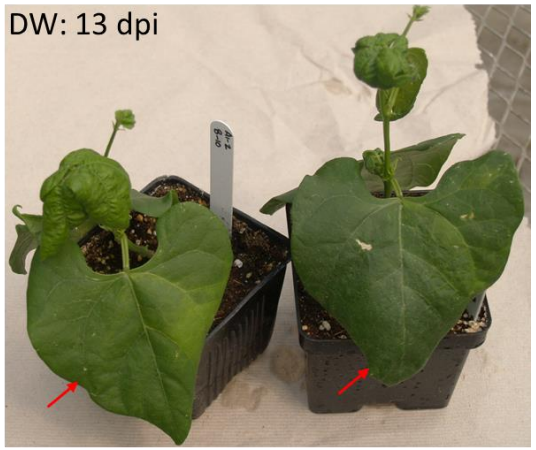
Idaho samples, common bean

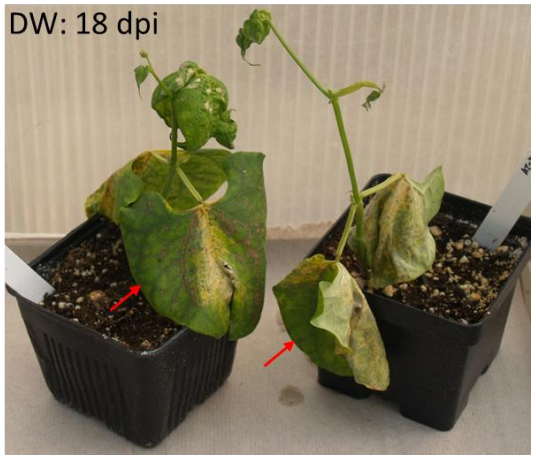
Name	ELISA		PCR					Sequence	
	BCMNV	BCMV	BCMNV	Poty-Deg II (HC-Pro)	Poty-Deg I (CI)	RU1	NL1	Ident., %	Match
F17:0298A	neg-	pos+	neg-	neg-	neg-	neg-	pos+	95%	PStV-JX014
F17:0298B	neg-	pos+	neg-	neg-	pos+	neg-	pos+	99%	NY15p
F17:0298C	neg-	pos+	neg-	neg-	pos+	neg-	pos+	99%	NY15p
F17:0298D	neg-	pos+	neg-	neg-	neg-	neg-	pos+	96%	PStV-JX014
F17:0298E	neg-	pos+	neg-	pos+	pos+	neg-	pos+	99%	NY15p

Conclusions

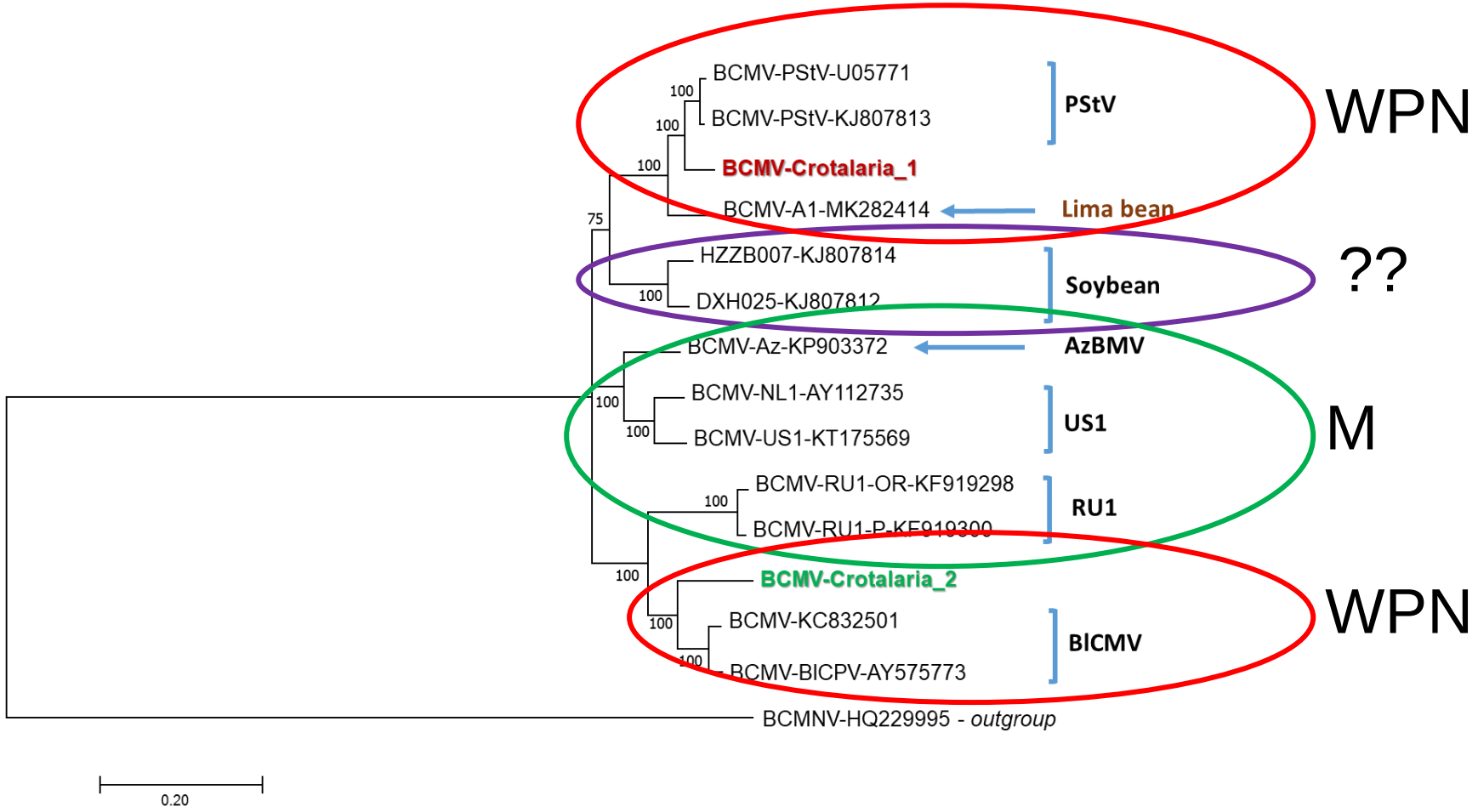
- We hypothesized that a novel BCMV strain, BCMV-A1, circulates in common bean in Idaho, perhaps in other states as well
- This new strain likely originated from lima bean
- It induced hypersensitive resistance and whole plant necrosis in some bean cultivars where it could spread systemically
- This new BCMV strain needs special tools to be identified and distinguished from conventional strains
- It needs to be incorporated in breeding programs to develop resistant cultivars

Similarities in biology of blackeye cowpea and lima bean strains of BCMV





BCMV-A1



Conclusions

- BCMV-C2 belongs to a non-*Phaseolus* lineage of BCMV strains, it belongs to the blackeye cowpea clade
- BCMV-C2 induces whole plant necrosis in common bean cultivars without resistance genes
- BCMV-C2 is able to overcome the *bc-u* gene and establish a systemic infection in common bean
- BCMV-C2 overcomes *bc-1* and *bc-2* in inoculated leaves only; *bc-1* and *bc-2* restrict its systemic movement
- BCMV-C2 is unable to overcome *bc-3* and *I* genes

Conclusions (continued)

- The role of *bc-u* in resistance to common bean strains of BCMV remains unclear
- In the absence of other resistance genes, *bc-u* suppresses the Whole Plant Necrotic reaction induced by BCMV-C2 in common bean
- This WPN reaction in common beans against BCMV-C2 (blackeye cowpea strain) and BCMV-A1 (lima bean strain) may be due to the expression of a yet unknown HR gene

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